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Honorable Editor-in-Chief
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Subject: Submission of an original research article for publication in your renowned journal.

Dear Sir,
With due respect, we would like to submit an original research article entitled **"Piper betel juice improved lipid profile and hepatic oxidative stress in high-fat-diet induced hyperlipidemic rats "** for publication in your widely circulated and renowned journal.

We declare that the manuscript contains original unpublished work and is not being under consideration for publication elsewhere.

We look forward for your cooperation in publishing the manuscript.

Thank you very much.

With great regards

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ORIGINAL RESEARCH ARTICLE

Piper betel juice improved lipid profile and hepatic oxidative stress in high-fat-diet induced hyperlipidemic rats

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Authors' contributions

MMRS conceptualized, designed, and supervised the study. XJ, SA, and AJG carried out the experiments. SA, FK, and MAS involved in data analysis, data presentation in tables and Fig.ures and interpreted data. XJ, SA, and AJG wrote the manuscript and MMRS reviewed it critically. All authors read the manuscript and agreed to be accountable for all aspects of the work and approved the final manuscript.

Running title: Betel leaf juice improved hyperlipidemia and oxidative stress in rats

Abstract

Background: *Piper betle* L., popularly known as "Paan" is a creeper plant which belongs to the genus Piper under the Piperaceae family. Leaves of this plant are very popular and traditionally used to chew with betel nut in many Asian countries. *P. betle* is also popular for its noteworthy ethnopharmacological values which ensure their use in folkloric medicine in different localities from the very ancient era.

Objectives: In this study, *P. betle* leaf juice (PBJ) was subjected to evaluate for its antihyperlipidemic activity on high-fat-diet induced hyperlipidemia in an experimental rat model.

Materials and Methods: Sprague-Dawley (SD) rats were allowed to high-fat diet *ad libitum* for a period of one month followed by concurrent administration of PBJ treatment along with high-fat diet for another one month. The rats were sacrificed at the end of the two months study for the evaluation and analysis of lipid profile and related parameters of hyperlipidemia.

Results: Our study showed a promising effect of PBJ on body weight, lipid profile, oxidative and antioxidative enzymes in the treated rats compared to the hypelipidemic control group. All doses of PBJ (0.5 – 3.0 mL/rat) significantly reduced the body weight of rats hyperlipidemic rats compared to hyperlipidemic control. PBJ at the doses of 1.0, 1.5, 2.0, and 3.0 mL/rat significantly ($p<0.05$, $p<0.01$, $p<0.001$) improved the levels of TC, LDL-c, TG, HDL-c and VLDL-c when compared with hyperlipidemic control group. Similarly, PBJ doses starting from 1.0 mL/rat to 3.0 mL/rat reduced the oxidative biomarkers of liver AST, ALT, ALP, and creatinine in comparison with hyperlipidemic control rats.

Conclusion: Our findings clearly demonstrated the potential lipid lowering activities of PBJ in hyperlipidemic rat model. Therefore, we can conclude that PBJ can be a good candidate for the development of antihyperlipidemic medication or as an alternative medicine.

Keywords:

Hyperlipidemia, Piper betel, *Piper betle* L, Betel leaves, Functional food, Nutraceutical, High fat diet, Oxidative stress, Sprague-Dawley (SD) rats

1. Introduction

Hyperlipidemia is characterized by the increment of normal levels of plasma lipids such as cholesterol, triglycerides, cholesterol esters, phospholipids and sometimes of plasma lipoproteins including very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) along with a decrement of high-density lipoprotein (HDL) levels.[1,2] Currently, it has become a widespread disorder and a threatening cause of many metabolic dysfunctions which may contribute to several prevalent diseases like heart diseases, atherosclerosis, high blood pressure, diabetes mellitus, hypercholesterolemia, obesity and so on [3,4] resulting in upraised rate of both morbidity and mortality incidence all over the world.[5] The extravagant consumption of dietary lipids gives rise to hyperlipidemia leading to the elevation of plasma cholesterol levels which in turns cause more than four million deaths yearly.[6,7] To treat this hyperlipidemic disease, life style modification approaches like less intake of fatty foods, giving up smoking, performing aerobic exercise, dietary therapies have been implemented along with the use of allopathic hypolipidemic drugs which can also alleviate the risks of other associated life-threatening disease states.[8,9] Both monotherapy and combination therapy of anti-hyperlipidemic drugs have demonstrated efficacy in the treatment of hyperlipidemia. Notably, five major classes of anti-hyperlipidemic drugs are Statins including lovastatin, pravastatin, simvastatin, atorvastatin, rosuvastatin; Bile acid binding resins like, cholestyramine and colestipol; Fibric acid derivatives including, gemfibrozil, bezafibrate, fenofibrate; Nicotinic acid derivatives like, niacin; and Cholesterol absorption inhibitors like, ezetimibe.[10] However, these commercially available anti-hyperlipidemic therapies like statins, deliver some major side-effects including gastrointestinal discomforts, rhabdomyolysis, myalgia, myopathy and dizziness which abate their credibility.[11] These side-effects are more prevalent at higher doses of statins.[10] In addition, these drugs can also cause kidney damage, cardiomyopathy along with an escalation in type 2 diabetes.[12,13] To compensate this, the usage of alternative treatments and therapies like herbal medicinal plants containing hypolipidemic constituents, have been increased and its popularity is rising day by day among both normal people and physicians across the world.[3] Thus, alternative medications such as consumption of lipid-lowering medicinal plants to attain antihyperlipidemic activity is observed in different localities. In addition, even in developed countries this approach

has been adopted in a large scale, especially when conventional drugs have failed to alleviate the disease state notably.[3]

Piper betel L., an evergreen and perennial creeper from the Piperaceae family is renowned for its heart shaped leaves.[14] It is native to Malaysia though cultivated in several other Asian countries including Bangladesh, India, Myanmar, Srilanka, Thailand, Vietnam etc.[15,16] Leaf of *P. betel*, the edible part of this plant is known as "Paan" with a minimum hundreds of varieties.[17] Betel leaf, having a pungent taste and distinct aroma due to the presence of phenols and terpenes is popularly used to chew with areca nut and slacked lime in many countries especially taken after having meals by local people as a mouth freshener. Its folkloric medicinal use includes wound healing and tonic properties.[18] In China, this plant is believed as a cure of several disorders including detoxification.[15] Besides, in Hindu culture, this leaf has profound importance in different social, cultural and religious events.[14] oil extracted from betel leaf is utilized as raw material of perfumes and food additives.[19] Betel leaf, an established wellspring of bioactive phytoconstituents exerts several pharmacological activities including antibacterial, anticancer, anthelminthic, antihypertensive, antiprotozoal, antifungal, antihistaminic, antifungal and antidiabetic properties.[14] Besides, this plant leaf is also popular in treatment of alcoholism, asthma, leprosy, bronchitis and dyspepsia.[20]

Phytochemical analysis of betel leaves provides an abundant source of carbohydrates, alkaloids, tannins, terpenoids, phenols, essential oils, and major other classes and isolated compounds include 1, 8-cineole, cadinene, camphene, caryophyllene, limonene, quercetin, pinene, chavicol, ally pyrocatechol, piperitol, carvacrol, safrole, eugenol, chavibetol, safrole, thymol, allyl eucalyptol, pyrocatechol monoacetate, eugenol, terpinen-4-ol, eugenyl acetate, quercetin etc.[21] However, antihyperlipidemic potential of this popular plant leaves has not yet been explored extensively. Therefore, we have aimed to conduct this study for the evaluation of *P. betel* leaf juice (PBJ) extract on high-fat-diet induced hyperlipidemia and hepatic oxidative stress in SD rats.

2. Materials and Methods

2.1 Materials

Betel (*Piper betel* L.) leaves were purchased from local sources in Bangladesh. The Cow's fat, dalda, cholesterol, ghee, coconut oil, and sodium cholate used in the high fat diet formulation were locally purchased. Ketamine HCl and Xylazine HCl were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other analytical grade reagents were locally procured and purchased. Simvastain as a standard lipid lowering drug was purchased from a local pharmacy.

2.2 Preparation of *Piper betel* L. leaves juice (PBJ)

The betel leaves were washed with distilled water and twenty-five (25) medium sized whole betel leaves with the pod (weight 113 g) were masticated and pressed in mortar and pestle to collect the juice in pressure. A total of 25 mL juice was collected from 113 g leaves. The PBJ was collected in a glass jar and stored at 4-8°C temperature from where it was used for experiment purposes.

2.3 Preparation of high fat diet

A high fat diet formulation was used for the induction of hyperlipidemia in Sprague-Dawley (SD) rats. The composition of high fat diet has been presented in Table 1. The cow's fat and dalda were melted with heat and gradually added to the normal pellets diet in a bucket. The liquids were mixed well with the pellets and allowed to become semi-solid/solid mass. Similarly, cholesterol and coconut oil were poured into the blended pellets and again homogenously mixed . Finally, coconut oil was added to the pellets. Thus, diet fat diet pellets were prepared and rats were provided with this high fat diet instead of the normal pellets diet.

2.4 Experimental animals

The experiments were conducted in SD rats, aged between 8-12 weeks. Sprague-Dawley rats having age of 6-8 weeks old were purchased from Shanghai Laboratory Animal Center (SLAC, Shanghai, China). The rats were housed six per plastic cage provided with wood chip bedding maintaining a 12 h light/dark cycle and allowed to

free access to standard rodent food and water *ad libitum*. Environmental changes were strictly controlled and prior to any experiment, the animals were kept for 1 week to adjust to the new housing environment.

2.5 Ethical Approval of Experimental Protocol

The ethical approval was obtained from the Animal Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University, Jinan, Shandong Province, China (Approval Number: 2020-031101) and Animal Ethics Committee of State University of Bangladesh, Dhanmondi, Dhaka, Bangladesh (Ethical approval No. 2020-02-03/SUB/A-AEC/0004). All the experiments were conducted according to the approved Animal Use Protocol by the Ethics Committee and following the rules, regulations, and laws of Shandong prefecture of China, maintaining the animal use rules and laws of Bangladesh, and in accordance with the Guidelines for Care and Use of Laboratory Animals published by the US National Institutes of Health. The Federation of European Laboratory Animal Science Associations (FELASA) guidelines and recommendations were followed to reduce the pain and stress of the experimental animals. At the end of the experiments, the rats were sacrificed with anesthesia overdose: Ketamine HCl (100 mg/kg) and xylazine (10 mg/kg) through intraperitoneal route.[22]

2.6 Acute toxicity study of PBJ

Oral acute toxicity study (LD_{50} determination) of *Piper betel* L. juice extract (PBJ) was performed following the OECD (Organization for Economic Cooperation and Development) by Fixed Dose Procedure (OECD protocol no. 420) as followed by Kifayatullah et al. (2015).[23] Briefly, Swiss Albino female (nulliparous and nonpregnant) rats, age: 8 weeks, were acclimized to laboratory conditions 7 days prior to experiment. The rats were divided into five groups, each comprising 5 animals. Group-1 served as the untreated control (received water only), group-2, 3, and 4 received PBJ doses 300 mg/kg, 2000 mg/kg, and 3000 mg/kg, respectively. The rats were overnight fasted for food (not fasted for water) before dosing and fasted for food 3-4 hours after the administration of doses. The animals were observed individually during the first 30 minutes after dosing, special attention was given during the first 4 hours, then to observe periodically during the first 24 hours to see any toxic effect in the animals.

During the entire period of observation for 14 days, the animals were observed and monitored for any changes in behavior, body weight, urinations, food intake, water intake, respiration, convulsions, tremor, temperature, constipation, changes in eye and skin colors and mortality of the animals.

2.7 Induction of hyperlipidemia

After the adaptation period, SD rats (7-9 weeks age) were fed with specially prepared high-fat diet (Table 1), consisting of 20% cow's fat, 6% dalda, 2% cholesterol, 2% coconut oil, 70% standard normal diet (w/w) for 1 month to induce hyperlipidemia. After 1 month, the rats were treated with PBJ along with the continuation of the high fat diet for another month.

Table 1. High Fat Diet Formulation

Sl. No.	Name of the ingredients	Quantity of ingredients	Percentage of ingredients
01	Cow's fat	600 g	20%
02	Dalda/Ghee	180 g	6%
03	Coconut oil	60 g	2%
04	Normal rats pellets diet	2.1 kg	70%
05	Cholesterol	60 g	2%
Total		3 Kg	100%

2.8 Experiment design

The rats were divided into the following eight (08) groups; each group will contain 5 rats:

Group-I: Normal control (rats received normal pellet diet and water, no treatment)

Group-II: Hyperlipidemic control (hyperlipidemic rats, no treatment)

Group-III: PBJ 0.5 mL/rat

Group-IV: PBJ 1 mL/rat

Group-V: PBJ 1.5 mL/rat

Group-VI: PBJ 02 mL/rat

Group-VII: PBJ 3 mL/rat

Group-VIII: Standard lipid lowering drug - Simvastatin (4 mg/kg)

The SD rats were treated every day for 30 days of the study period.

2.9 Determination of lipid profile and oxidative biomarkers in rats

At the end of the experiment period, blood was collected by cardiac puncture from the animals using anesthesia. The blood was then centrifuged at 3000 rpm for 15 min, and the serum was stored at -80 °C for analysis. The amounts of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL-c), high-density lipoprotein (HDL-c), and apolipoprotein B-48 (apoB48) were measured by using commercial assay kits according to the manufacturer's instructions (Roche, Basel, Switzerland). Other biomarkers which include aspartate transaminase (AST), alanine transaminase (ALP), alkaline phosphatase (ALP), and serum creatinine were determined following the standard protocol [24].

2.10 Determination of body weight of rats

Body weights of rats were measured before starting the experiments, one month after high fat diet, and at the end of the experiments in which the rats were treated with PBJ for another one month along with a high fat diet.

2.12 Histopathological evaluation of liver

Histopathological experiments were performed as described by Song et al. (2018).[25] After sacrificing the rats, livers were collected and immediately fixed in 10% buffered formalin (pH 7.4) and embedded in paraffin. A portion of the liver was cut (4–5 μm), stained with haematoxylin-eosin (H&E), and the sections were examined with a computer-aided microscope for the determination of morphological and/or pathological changes (×600 magnification). The histological scores were assessed as mentioned by Ishak et al (1995).[26] scoring systems were performed as followed by Yahya et al. (2013) [27] based on a sum of three parameters: inflammation grade, cell infiltration, and tissue disruption. H&E staining in the liver was scored using a scale of 0 to 4 (0 = no inflammation grade, cell infiltration, and tissue disruption; 1 = 0–25% inflammation grade, cell infiltration and tissue disruption; 2 = 25–50% inflammation grade, cell infiltration and tissue disruption; 3 = 50–75% inflammation grade, cell infiltration and

tissue disruption; and 4 = 75–100% inflammation grade, cell infiltration and tissue disruption). Each tissue was evaluated for the sum of three parameters and by the degree of liver injury using a qualitative score that ranged from 0 to 4. A score of 0 was categorized as no damage, scores between 1 and 2 were categorized as light injury, and scores of 3 and 4 were categorized as serious injury.

2.13 Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) using Statistical Package SPSS software (version 25.0) of IBM (International Business Machines) Corporation, USA, followed by Dunnett's-T3 and Tukey HSD tests to determine statistical significance between groups. The data are means ± S.E.M. (standard error mean) of five animals with 95% confidence intervals (CI). The p-value, $p< 0.05$ was considered as statistically significant.

3. Results

3.1 Effect of PBJ on the body weight of hyperlipidemic rats

Table 2 summarizes the effect of PBJ in hyperlipidemic rats model and showed a notable effect on the reduction of body weight. The body weight of experimental rats increased remarkably ranging from 271.6 gm to 302.8 g after one-month continuation of high-fat diet in the hyperlipidemic control group (HC) compared to the non-high fat diet control (NHC) group which showed a mean body weight of only 183.8 g. Rats were further provided with high-fat diet for another month along with the treatment with different doses of PBJ (0.1 mL/rat - 3.0 mL/rat) and the weights were re-documented. As presented in Table 2, we can see that the hyperlipidemic control rat weight (319.00 ± 7.127) has significantly increased due to the exposure of rats with high fat diet compared to the weight (186.00 ± 3.563) of non-hyperlipidemic control group (NHC) (rats of normal diet) (ϕϕϕ $p<0.001$). On the contrary, all five groups of rats treated with five different doses of PBJ (0.5, 1.0, 1.5, 2, and 3 mL/rat) exhibited prominent (***$p<0.001$) abatement in body weights comparing to the hyperlipidemic control rats. Simvastatin (4 mg/kg), used as a standard lipid lowering drug group also exhibited a potential reduction of body weight (206.4 ± 3.429 g) when compared with hyperlipidemic control (***$p<0.001$).

Table 2. Effect of PBJ on body weight of rats

Treatment group	Body weight in gram		
	Body weight before high fat diet	**Body weight 1 month after high fat diet**	**Body weight after 1 month treatment of hyperlipidemic rats**
Non-hyperlipidemic Control (NHC)	174.2	183.8	186.00 ± 3.563
Hyperlipidemic control (HC)	161.8	288.4	319.00 ± 7.127 ϕϕϕ
Piper betle L. juice (PBJ) 0.5 mL/rat	178	291.2	197.00 ± 6.607 ***
Piper betle L. juice (PBJ) 1.0 mL/rat	177.8	291.4	220.00 ± 7.69 ***
Piper betle L. juice (PBJ) 1.5 mL/rat	163.8	271.6	200.00 ± 5.54 ***
Piper betle L. juice (PBJ) 2.0 mL/rat	182.6	294.6	210.00 ± 5.357 ***
Piper betle L. juice (PBJ) 3.0 mL/rat	170.2	295.8	218.6 ± 6.867 ***
Simvastatin (4 mg/kg)	184.2	302.8	206.4 ± 3.429 ***

Values are expressed as means ± S.E.M. of five rats in each group.

* Data differed significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) when compared to hyperlipidemic control (HC) group

ϕ Data differed significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) when compared to non-hyperlipidemic control (NHC) group

3.2 Effect of PBJ on the lipid profile in hyperlipidemic SD rats

The effect of PBJ on lipid profile in hyperlipidemic rats has been presented in Fig. 1. The normal values of lipids in rats are TC 113.99±2.18 mg/dL, LDL-c 49.64±1.82

mg/dL, TG 76.13 ± 2.38 mg/dL, HDL-c 49.14±1.05 mg/dL, VLDL-c 15.22 ±0.48 mg/dL.[27] Treatment of hyperlipidemic rats with PBJ at the doses of 1.0, 1.5, 2.0, and 3.0 mL/rat potentially reduced the total cholesterol levels compared to hyperlipidemic control (HC) rats group (Fig.1A). Similarly, PBJ (1.0, 1.5, 2.0 and 3.0 mL/rat) significantly lowered the levels of low density lipoprotein (LDL-c) (Fig. 1B), total cholesterol (TG) (Fig. 1C) and very low density lipoprotein cholesterol (VLDL-c) (Fig. 1E) comparing to that of hyperlipidemic control. Additionally, PBJ at the doses of 1.5, 2.0, and 3.0 mL/rat has improved the high-density liproprotein with the increment of the levels of HDL-c in hyperlipifemic rats (Fig. 1D).

Fig. 1(A)

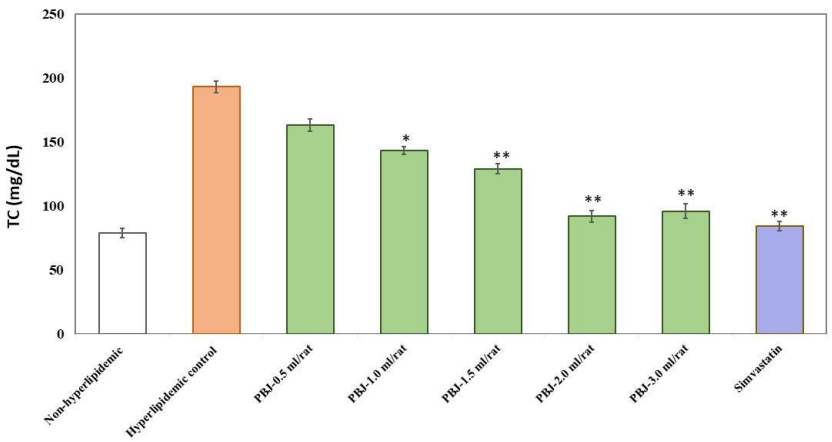


Fig. 1(B)

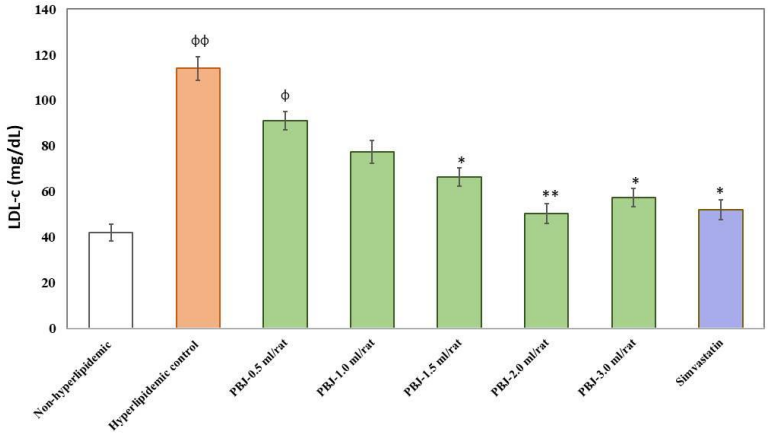


Fig. 1(C)

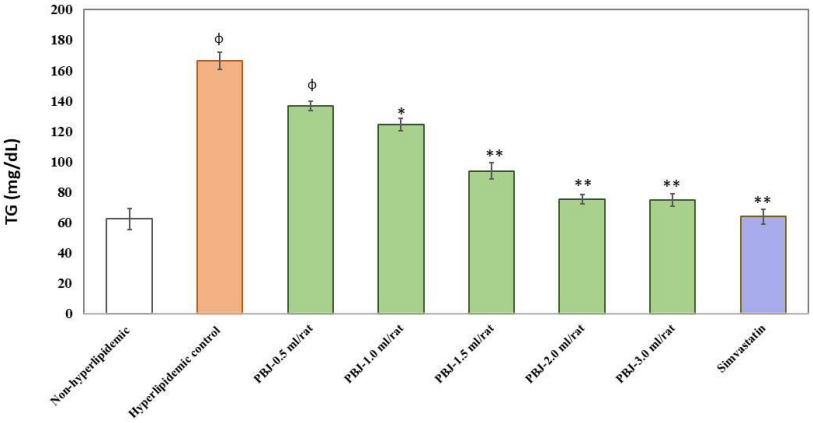


Fig. 1(D)

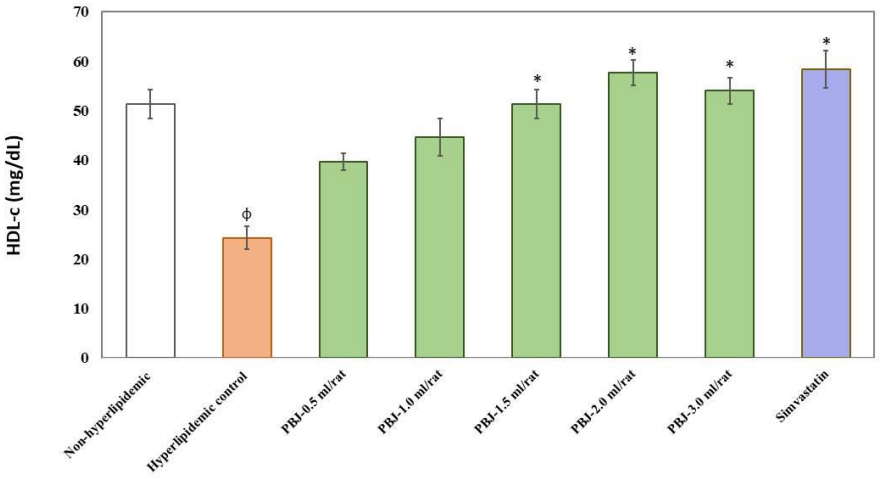
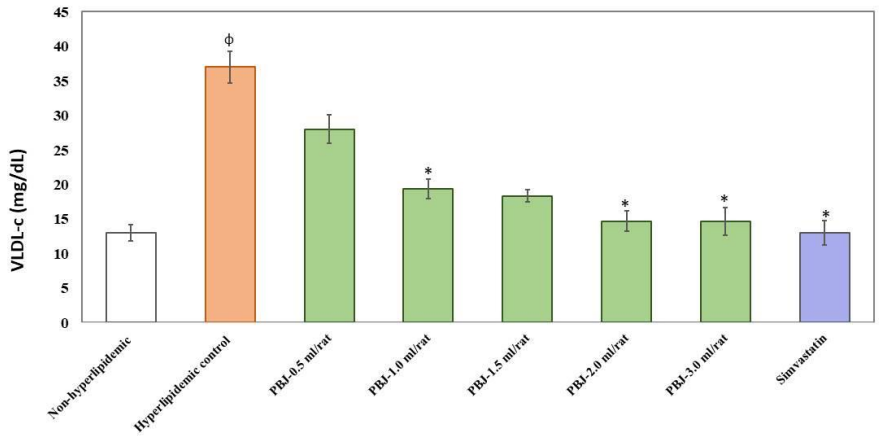


Fig. 1(E)



3.3 Effect of PBJ on oxidative and antioxidant enzyme activities

Figure 2 (A-D) demonstrates the positive effect of PBJ on biochemical markers in hyperlipidemic rats model. The normal value of AST is 50-150 IU/L, ALT is 10-40 IU/L, ALP is 30-130 IU/L, and Creatinine is 0.25 – 3.09 mg/dL in rats. [28,29] As shown in the Fig. 2, high fat diet in rats drastically enhanced the levels of AST (131.0 ± 5.507 U/L vs. 61.0 ± 5.507 U/L), ALT (81.0 ± 5.507 U/L vs. 38.0 ± 1.732 U/L), ALP (165.67 ± 5.783 U/L vs. 87.0 ± 4.932 U/L) and creatinine (1.933 ± 0.049 mg/dL vs. 0.686 ± 0.095 mg/dL), respectively. However, treatment of hyperlipidemic rats with different doses of PBJ 0.5 to 3.0 mL/rat significantly diminished the levels of different biomarkers: AST, ALT, ALP, and creatinine (*p<0.05, **p<0.01, ***p<0.001). Simvastatin as a standard antihyperlipidemic drug at the dose of 4 mg/kg also similarly diminished the levels of AST, ALT, ALP, and creatinine.

Fig. 2(A)

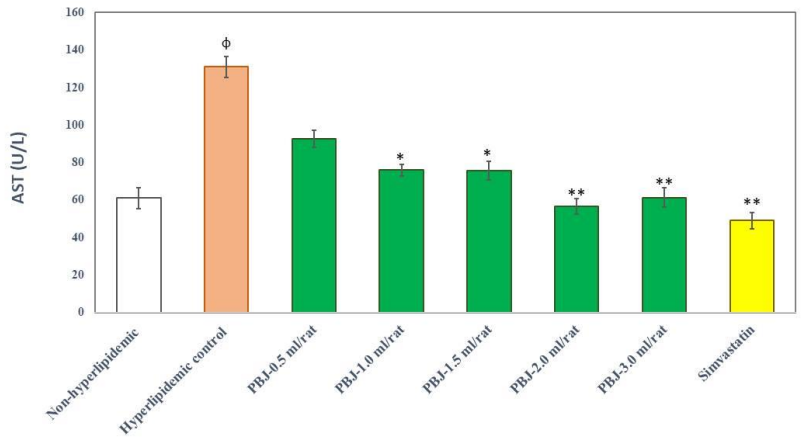


Fig. 2(B)

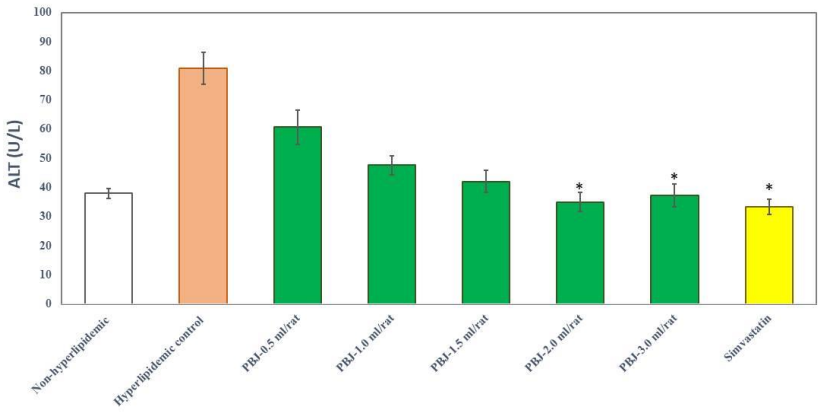


Fig. 2(C)

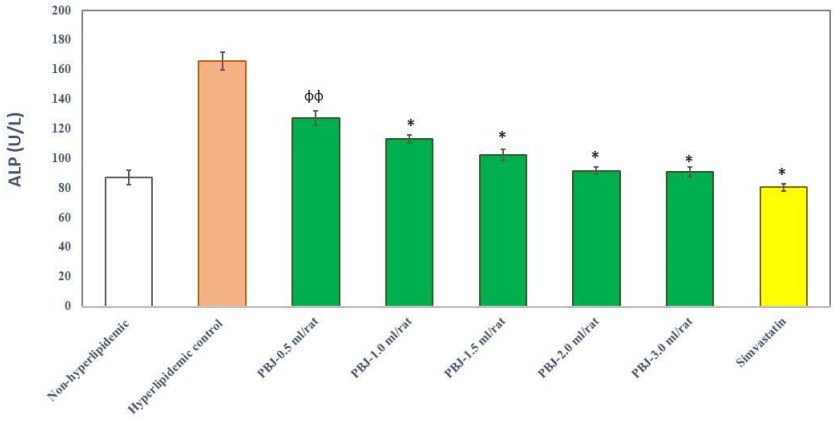
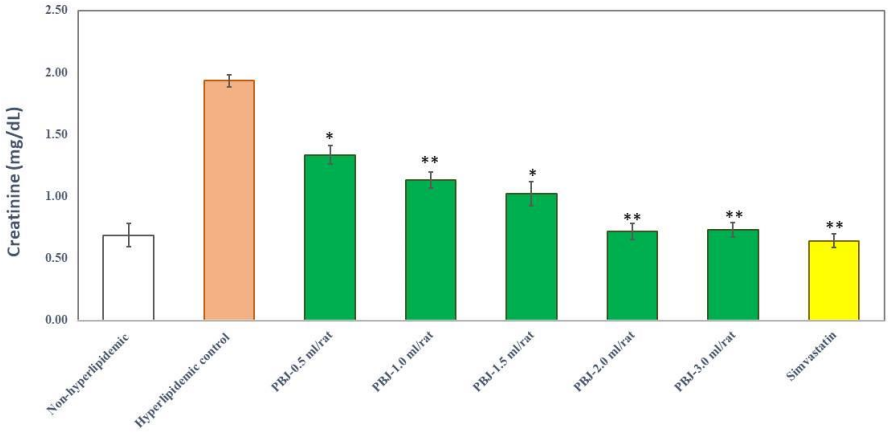


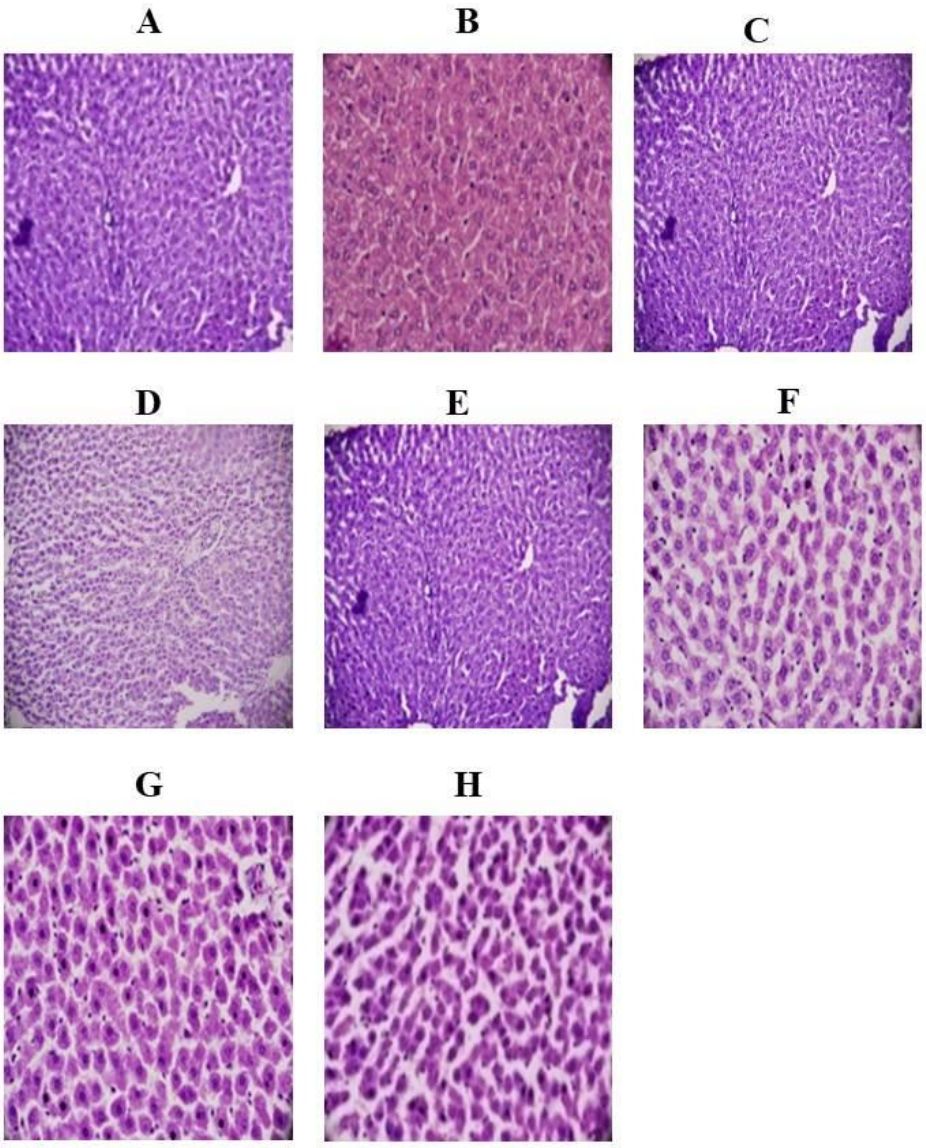
Fig. 2(D)



3.4 Effect of PBJ on histopathological assessment of high fat diet-induced liver damage

Histopathological observation of liver sections of non-hyperlipidemic control group showed normal cellular architecture, distinct hepatic cells, sinusoidal spaces, central vein, and no accumulation of fat (Fig. 3A). In high-fat-diet group, hepatic cells were found to have hyperplasia, cellular degeneration, inflammation, and accumulation of fat (Fig. 3B). The rats treated with PBJ (0.5, 1.0, 1.5, and 2 mL/rat) (Fig. 3C, D, E, F, G) and Simvastatin 4 mg/kg (Fig. 3H) showed almost normal architecture of the hepatic cells having signs of protection with the reduced number/absence of inflammatory cells, vascular degeneration and significance reduction/absence of fatty cells accumulation.

Fig. 3



3.5. Oral acute toxicity study of PBJ

Oral acute toxicity study of PBJ was carried out following OECD guideline Fixed Dose Procedure (OECD protocol no. 420) as mentioned in the Methodology section. No case of mortality was observed during the 14 days of treatment with a limited dose of 5000 mg/kg BW of PBJ. All treated animals could tolerate the PBJ doses and there was no statistically significant difference in body weight between the treated and untreated groups. The animals did not exhibit any abnormalities or major behavioural changes such as respiratory distress, abnormal locomotion, tremors, salivation, diarrhoea, sleep,

walking backwards, reaction to handling, catalepsy, coma or any toxic symptoms either immediately or during the posttreatment observational period of 14 days. Thus, we can say that the LD_{50} for oral administration of PBJ is higher than 5000 mg/kg B.W. and is apparently nontoxic. Therefore, the used doses of PBJ (50-800 mg/kg) were well tolerated by the animals (data not shown).

4. Discussion

High-fat diet can increase cholesterol level in susceptible individuals which in turn results in hyperlipidaemia and obesity.[30] Besides, hyperlipidaemia, a severe risk factor of coronary complications is considered as a prominent aetiology of early death throughout the world.[31] Previous studies also reported that an elevated level of HDL-c and diminished level of TG, TC and LDL-c alleviate cardiac health by preventing ischemic condition.[32] The action of antihyperlipidemic agents can be attributed by improving cessation of intestinal cholesterol absorption, catabolism of body cholesterol, interfering with the production of lipoprotein along with enhanced expression of LDL-c receptors in the liver and their protection and elimination of LDL-c from the blood by promoting their degradation. However, all these episodes either collectively or independently abridged LDL-c levels resulting in mitigation of TC concentration in blood during the treatment with sample extracts.[33-35]

This present study was conducted to investigate the role of *P. betel* leaf juice in high-fat diet induced hyperlipidaemic conditions. High-fat diet significantly increased TG, TC, LDL-c, and VLDL-c levels as expected in rat models along with a prominent reduction in HDL-c levels compared to the normal physiologic range. In this experiment, when the *P. betel* left juice was coadministered with the high-fat diet, it delivered very promising responses in all parameters which were very closely comparable to the standard drug, simvastatin. Histopathological observation of liver sections was also documented the efficacious role of *P. betel* juice in experimental rat models. In addition, previously conducted phytochemical analysis of P. *betel* revealed several phytochemicals including polyphenols, by which it may exert its noteworthy benevolent activity by alleviating the hyperlipidaemia state. [36], This observation also ascertains the claim of considering *P. betel* as a promising candidate of drug discovery or use the betel leaves juice preparations for the treatment of hyperlipidaemia.

5. Conclusion

The conducted study on high fat diet-induced hyperlipidemia in rat model provides some important insights about the antihyperlipidemic activity of *P. betel* leaf juice by evaluating biochemical and histopathological parameters. The findings of our study clearly reveals a potential lipid lowering activities of *P. betel* which were very close to standard drug available in market and gives a ray of hope to consider it as a possible wellspring of hypolipidemic drug agents or the PBJ can be directly used as an alternative medicine for the treatment of hyperlipidemia. However, further studies are requested to establish the responsible active compounds and their possible mode of action as well as a complete safety profile.

Consent of publication

All authors of this manuscript have consented to publish the article and they don't have any conflict of interest on this article.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study have been included in the article and its supplementary files.

Competing interests

The authors declare that they have no competing interests in the research work and to publish the article.

Ethical approval

The ethical approval for the experimental protocol of animal care and use in this study was obtained from the Animal Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University, Jinan, Shandong Province, China (Approval Number: 2020-031101) and Animal Ethics Committee of State University of Bangladesh, Dhanmondi, Dhaka, Bangladesh (Ethical approval No. 2020-02-03/SUB/A-AEC/0004).

Statement of Human and Animal rights

All experiments involving animals were conducted according to the approved Experimental Animal Care and Use Protocol by the Animal Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University, China, and in accordance with the Guidelines for Care and Use of Laboratory Animals published by the US National Institutes of Health. The Federation of European Laboratory Animal Science Associations (FELASA) guidelines and recommendations were followed to reduce the pain and stress of the experimental animals.

Statement of informed consent

There are no human subjects in this article and informed consent is not applicable.

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Figure Legends

Fig.1. Effect of PBJ on TC (Fig. A), LDL-c (Fig. B), TG (Fig. C), HDL-c (Fig. D) and V-LDL-c (Fig. E) in rats. SD rats were given high-fat diet for a month followed by another one month of concurrent administration of treatment and high-fat diet. Values are expressed as means ± S.E.M. of five rats in each group. *Data differed significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) when compared to hyperlipidemic control (HC) Group. ϕ Data differed significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) when compared to non-hyperlipidemic control (NHC) group

Fig. 2. Effect of PBJ on AST (Fig. A), ALT (Fig. B), ALP (Fig. C) and creatinine (Fig. D) in rats. SD rats were given high-fat diet for a month followed by another one month of concurrent administration of treatment and high-fat diet. Values are expressed as means ± S.E.M. of five rats in each group. *Data differed significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) when compared to hyperlipidemic control (HC) Group. ϕData differed significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) when compared to non-hyperlipidemic control (NHC) group

Fig. 3. Representative photomicrographs of liver histopathology. Liver sections from SD rats group of non-hyperlipidemic control (A), hyperlipidemic control (B), PBJ 0.5 mL/rat (C), PBJ 1.0 mL/rat (D), PBJ 1.5 mL/rat (E), PBJ 2.0 mL/rat (F), PBJ 3.0 mL/rat (G), and Simvastatin 4 mg/kg (H), respectively.